First report of an mcr-1-harboring Salmonella enterica subsp. enterica serotype 4,5,12:i:- strain isolated from blood of a patient in Switzerland

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First report of an *mcr-1* harbouring *Salmonella enterica* subsp. *enterica* serovar 4,5,12:i:- strain isolated from blood of a patient in Switzerland

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Sir,

In the past, the use of colistin has been mostly limited to veterinary medicine due to its rather severe side effects, but given the increase in multi-resistant Gram-negative bacteria, the WHO recently relabelled colistin as a "critically important antibiotic". The first description of a plasmid-borne mobilized colistin resistance gene *mcr-1* in 2015[1] caused great concern, as the ease of potential spread on a conjugative plasmid encoding resistance to polymyxins might change the resistance situation to colistin drastically. In line with this, *mcr-1* mediated colistin resistance in Enterobacteriaceae has since been reported from a wide range of geographical locations[2,3]. Here, we report the first case of *mcr-1*-harbouring *Salmonella enterica* in Switzerland.

Salmonella enterica subsp. *enterica* serovar 4,5,12:i:- strain N17-0346 was isolated in 2017 from the blood of a 77-year-old male patient in Switzerland with no known travel history.

The strain was subjected to susceptibility testing against 16 antimicrobial agents by the disc diffusion method according to CLSI protocols and evaluated according to CLSI criteria. Determination of the minimum inhibitory concentration (MIC) of colistin was performed by broth microdilution according to the European Committee on Antimicrobial Susceptibility Testing EUCAST (eucast.org). The strain N17-0346 was phenotypically resistant to colistin (MIC=4 mg/L), ampicillin, and streptomycin. It was sensitive to amoxicillin with clavulanic acid, cefazolin, cefotaxime, cefepime, gentamicin, kanamycin, nalidixic acid, ciprofloxacin, sulfamethoxazole with trimethoprim, azithromycin, fosfomycin, nitrofurantoin, tetracycline and chloramphenicol.

The strain was sequenced using the MiSeq platform (Illumina, San Diego, CA, USA) and a Nextera XT library kit utilizing either 500 or 600 cycles of paired-end reads (Illumina). The paired end libraries were generated and sequenced in conjunction with the Nextera XT DNA sample preparation guide on the Illumina Miseq instrument (Illumina; San Diego, CA). *De*

novo assembly with CLC Genomics Workbench version 9.0 (CLC bio, Aarhus, Denmark) resulted in a genome size of 4,981,418 bp, with 95 contigs and a GC content of 52.1 %. The genome was annotated using the RAST annotation server, and 5,061 coding sequences were identified. *In silico* seven-gene multilocus sequence typing (MLST) using seq2mlst v. 1.0.1 (https://github.com/lmc297/seq2mlst) identified N17-0346 as ST34. Core-genome MLST using the command-line implementation of SISTR v. 1.0.2 classified it as ST 3833327333. Genome-wide detection of antimicrobial resistance (AMR) genes using the method described and validated for *S.* Typhimurium by Carroll et al.[4] and implemented in BTyper v. 2.2.0 in conjunction with the ARG-ANNOT AMR gene database, PlasmidFinder, and PlasFlow v. 1.0 produced the following hits: *mcr-1* encoded on an IncX4 plasmid with no further antibiotic resistance genes on the same contig, *strA/strB* (conferring streptomycin resistance) and *sulII* (encoding sulfonamide resistance) were located on an IncQ1 plasmid, $bla_{\text{TEM-30}}$ (coding for an inhibitor-resistant β-lactamase) on a contig classified as belonging to a plasmid, and *aac(6')-Iaa* (aminoglycoside acetyltransferase) encoded on the chromosome.

The *mcr-1* coding sequence was located directly upstream of an open reading frame encoding a hypothetical protein with similarities to a PAP2 superfamily protein that is frequently seen in associated with *mcr-1*[5]. A single copy of an incomplete version of insertion sequence IS*Apl1* was located downstream of the *mcr-1* cassette, but no IS*Apl1* element was identified upstream of it.

Conjugal transfer of the *mcr-1* harbouring IncX4 plasmid from N17-0346 as the donor strain to *E. coli* HK225 (streptomycin and rifampicin resistant) as the recipient strain was tested at 25 and 37 °C, in liquid medium as well as on solid agar plates. Transconjugants carrying the *mcr-1* gene as confirmed by PCR were found in all of these experiments, with a conjugation efficiency of 2.2×10^{-4} on solid agar at 37°C. To our knowledge, this is the first report of a *Salmonella* strain carrying an *mcr-1* colistin resistance gene in Switzerland.

The full sequence of strain N17-0346 has been deposited in GenBank under the accession no. QEAL0 0 0 0 0 0 0 0 and the sequence of the *mcr-1* -harboring plasmid pN17-0346 under accession no. NZ _ CP031291.1.

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Declarations

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Competing Interests: None

Ethical Approval: Not required

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